Genomic Editing of the HIV-1 Coreceptor CCR5 in Adult Hematopoietic Stem and Progenitor Cells Using Zinc Finger Nucleases.

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Public Summary:
It is well known that infection with HIV-1 requires a protein called CCR5. Persons with a natural mutation in this gene (CCR5Δ32) are protected from HIV/AIDS. In an effort to control HIV-1 in an infected person, our goal is to develop a method to inactivate the CCR5 gene, which would possibly result in less spread of HIV-1 and potentially no need for continued anti-HIV-1 medications. Toward this goal, we used proteins that are called Zinc Finger Nucleases (ZFNs) that can disrupt the CCR5 gene in blood stem cells. With these ZFNs, we engineered a person's own blood stem cells (called autologous CD34+ cells) and then tested whether this method efficiently disrupted the CCR5 gene. We found that the method did efficiently modify the CCR5 gene; using special laboratory conditions, we achieved more than 25% CCR5 gene disruption in the stem cells. We then showed that these cells could still be transplanted and make an immune system in a mouse. These results establish a basis for a new approach exploiting a ZFN delivery system to modify the CCR5 gene of blood stem cells, and this method has potential as a future anti-HIV-1 treatment.

Scientific Abstract:
The HIV-1 coreceptor CCR5 is a validated target for HIV/AIDS therapy. The apparent elimination of HIV-1 in a patient treated with an allogeneic stem cell transplant homozygous for a naturally occurring CCR5 deletion mutation (CCR5Delta32/Delta32) supports the concept that a single dose of HIV-resistant hematopoietic stem cells can provide disease protection. Given the low frequency of naturally occurring CCR5Delta32/Delta32 donors, we reasoned that engineered autologous CD34+ hematopoietic stem/progenitor cells (HSPCs) could be used for AIDS therapy. We evaluated disruption of CCR5 gene expression in HSPCs isolated from granulocyte colony-stimulating factor (CSF)-mobilized adult blood using a recombinant adenoviral vector encoding a CCR5-specific pair of zinc finger nucleases (CCR5-ZFN). Our results demonstrate that CCR5-ZFN RNA and protein expression from the adenoviral vector is enhanced by pretreatment of HSPC with protein kinase C (PKC) activators resulting in >25% CCR5 gene disruption and that activation of the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway is responsible for this activity. Importantly, using an optimized dose of PKC activator and adenoviral vector we could generate CCR5-modified HSPCs which engraft in a humanized mouse model (albeit at a reduced level) and support multilineage differentiation in vitro and in vivo. Together, these data establish the basis for improved approaches exploiting adenoviral vector delivery in the modification of HSPCs.Molecular Therapy (2013); doi:10.1038/mt.2013.65.